PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	Docket No: Q75484
Rakesh TULI, et al.	
Application No.: 10/661,478	Group Art Unit: 1636
Confirmation No.: 9837	Examiner: Joike, M.
Filed: September 15, 2003	

For: CHEMICALLY SYNTHESIZED ARTIFICIAL PROMOTER FOR HIGH LEVEL EXPRESSION OF TRANSGENES AND A METHOD FOR ITS SYNTHESIS

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Samir V. Sawant, hereby declare and state:

THAT I am a citizen of India;

THAT I have received the degree of Ph.D in Biochemistry from Lucknow University;

THAT I have been employed by NBRI, CSIR since Jan 2002, where I hold a position as Scientist, with responsibility for Plant Molecular Biology;

I further declare and state as follows.

THAT I have reviewed the Office Action mailed December 20, 2006, and, in particular, the Examiner's contention as to claims 20, 21, 26, 29 and 47 that the claims read on a broad genus of promoters and functional variants thereof but allegedly do not provide sufficient description of a representative number of species.

Claim 20 now reads as follows:

20. (currently amended) A promoter comprising at least one of each of the following elements (i)-(ix), or functional fragments thereof, in the 5' to 3' direction:

(i) domain II which comprises at least one member selected from the group consisting of subdomain II (a), subdomain II (b), subdomain II (c), subdomain II (d) and domain III, wherein subdomain II (a) is SEQ ID NO: 7, or subdomain II (a) is a functional sequence with at least 50% sequence identity to SEQ ID NO: 7 and activates transcription;

(ii) domain I, which comprises at least one member selected from the group consisting of subdomain I (a), subdomain I (b), and subdomain I (c), wherein subdomain I (a) is SEQ ID NO: 18, or subdomain I (a) is a functional sequence with at least 75% sequence identity to SEQ ID NO: 18 and activates transcription; ;

(iii) minimal domain (b), wherein minimal domain (b) is SEQ ID NO: 5, or minimal domain (b) is a functional sequence with at least 75% sequence identity to SEQ ID NO: 5 and activates transcription;

(iv) minimal domain (a), wherein minimal domain (a) is SEQ ID NO: 2, or minimal domain (a) is a functional sequence at least 75% homologous to SEQ ID NO: 2 and activates transcription ;

(v) region between minimal promoter (a) and a transcription start site, wherein said region between minimal promoter (a) and said transcription start site is SEQ ID NO: 12, or said region between minimal promoter (a) and the transcription

start site is a sequence with at least 75% sequence identity to SEQ ID NO: 12;

(vi) transcription start site, wherein said transcription start site is SEQ ID NO: 4, or said transcription start site is a functional sequence with at least 50% sequence identity to SEQ ID NO: 4 and activates transcription;

(vii) 5' untranslated leader region, wherein said 5' untranslated leader region is SEQ ID NO: 13, or said 5' untranslated leader region is a sequence with at least 75% sequence identity to SEQ ID NO: 13;

(viii) translational initiation codon, wherein said translational initiation codon is SEQ ID NO: 14, or said translational initiation codon is a sequence with at least 75% sequence identity to SEQ ID NO: 14; and

(ix) a polynucleotide encoding the amino acid sequence set forth in SEQ ID NO. 16.

As shown by the following experiments, the specification of the above-identified application enables a person of ordinary skill in the art to identify and create all species encompassed by the claims. In the following experiments, prototype 13-bp TATA-box sequence, TCACTATATATAG, was mutated at each nucleotide position and examined for its function in the core promoter. Specific nucleotides in the first TATA, the second TATA, as well as the flanking sequences influenced promoter function in transient transformation of tobacco leaves. The experiments give direct evidence for the role of a core TATA-box sequence in determining the level as well as selectivity of gene expression in plants by mutating, at every possible position, the core promoters encompassed by Applicants' claims.

MATERIALS AND METHODS

We have previously reported (Sawant et al., 1999) TCACTATATATAG as the most commonly present TATA-box sequence in highly expressed plant genes. The functional validity of this sequence has been established previously by demonstrating that an upstream region activates transcription dependent upon the core promoter containing this TATA-box sequence. Furthermore, the TATA-box sequence functioned at a high level in a number of plant species and in different plant tissues (Sawant *et al.*, 2000).

Mutations at each of the 13 positions in the prototype TATA-box, TCACTATATATAG, were created by PCR using degenerate primers. (Please refer to Kiran *et al.* preprint, attached herewith and incorporated herein in its entirety by reference.) Briefly, a total of 13 degenerate primer sets were synthesized. A given primer set represented the three possible nucleotide substitutions at a specific position. The PCR products were cloned into a pUC19 (New England Biolabs) vector. A total of 10 random clones were picked for each primer and sequenced. A total of 39 mutations in the 13-bp-long prototype TATA-box sequence were selected. Representative double mutations were also created by a second cycle of PCR mutagenesis (as indicated). Gene expression was determined using reporter gene constructs as indicated at pages 11 and 12 of Kiran *et al.*

RESULTS

Our experiments reveal that several single mutations in the TATA-box region are tolerated *in vivo*. The effect of single mutations in the TATA-box on transient gene expression from reporter gene constructs was observed by determining the activity after incubating leaf discs for 48 h in continuous light. The GUS activity expressed by the prototype TATA-box, TCACTATATATAG, was compared with the activity of the mutated derivatives following incubation of the excised leaves in light. As seen in Figure 1, page 3 of Kiran *et al.*, although several mutations had no significant effect on the activity of the basal promoter, a majority of mutations resulted in a decrease or an increase in promoter expression as compared to the prototype promoter that contained the parent TATA sequence.

For example, a 5-fold increase in light was obtained when G at the thirteenth position was mutated to C (G13/C). Our experiments on transient transformation of tobacco leaves show that, in contrast to in vitro transcription, mutations in the core TATA sequence are more tolerated in vivo. In the case of mutations like T9/A, no significant effect was observed by us, which is in agreement with that reported in an in vitro tobacco-based transcription system (Yamaguchi et al.,1998). Several mutations, especially those at the fifth, seventh, eighth, and tenth positions, and others like A3/G, C4/G, A12/C, etc., decreased transcription substantially. However, others, especially at the eleventh and thirteenth positions, had a substantial increase in activity in light. The effect of mutations on gene expression suggested the importance of both TATATATA as well as the flanking sequences (*i.e.* the TCAC and G) located, respectively, before and after it, in determining the level of in vivo transcription.

To our knowledge, these flanking positions have not been studied with respect to modulation of promoter expression. Mutations that were not tolerated at all in gene expression in leaves exposed to continuous light were the substitutions to C and G at the seventh and eighth positions (*i.e.*, the latter TA of the first TATA sequence). The promoters containing a TATA-box with these mutations failed to activate gusA expression in leaves exposed to continuous light. Figure 1. The results suggest that certain positions in the prototype TATA-box have very little or no sequence preference for in vivo promoter function, although others could be critical to the level of gene expression.

CONCLUSIONS

Applicants herewith provide direct evidence of numerous promoters encompassed within the scope of Applicants' claims by illustrating, in great detail, complete mutation and corresponding transcriptional activity consistent with the methods described in Applicants' specification. The variety of species disclosed in this Declaration and accompanying report encompass the claimed embodiments of Applicants' invention and therefore represent an actual reduction to practice of all species encompassed by the claims.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 19/6/2007

{NAME} Dr. Samir V. Sawant